

Meat Safety News Digest

A collection of recent news relevant to the safety of red meat prepared by the Food Safety Program of Meat & Livestock Australia, for SAFEMEAT Stakeholders

INTERVENTIONS

Identification of *E. coli* O157:H7 surrogate organisms to evaluate beef carcass intervention treatment efficacy

This USA study compared survival of potential pathogen surrogates (lactic acid bacteria, indigenous indicator organisms [non-*E. coli* coliforms, biotype I *E. coli*]) with the survival of pathogenic *E. coli* O157:H7 in beef carcass interventions at abattoirs. Surrogates are non-pathogenic organisms that, when added to a food product, behave similarly to the target pathogen when exposed to processing conditions, and are easily enumerated. The experiments were performed using beef brisket (adipose and lean), cod fat membrane, or neck tissue. Intervention treatments were grouped by abattoir size: small (6-day dry aging; 22°C acid treatment, 1-day dry aging; hot water) and large (warm acid treatment with or without a preceding hot water treatment). A surrogate pathogen was considered a suitable replacement for *E. coli* O157:H7 if the intervention produced a reduction in surrogate levels that was not significantly greater than that observed for *E. coli* O157:H7. All three surrogate inocula were suitable for dry-aging and acid spray plus dry-aging treatments used by small abattoirs. No one inoculum was suitable as a surrogate across all intervention treatments used by large abattoirs. Hence, the convenience of using a single-isolate surrogate weighed against the versatility of using multi-isolate

surrogates is an important factor to consider in using these organisms. Overall, the results of the study supported the concept of using non-pathogenic surrogate organisms to validate beef carcass intervention treatments for efficacy against *E. coli* O157:H7.

<http://www.ingentaconnect.com/content/iafp/jfp/2010/00000073/00000010/art00012>

Validation of lactic acid bacteria, lactic acid, and acidified sodium chlorite as decontaminating interventions to control *E. coli* O157:H7 and *Salmonella* Typhimurium DT 104 in mechanically tenderized and brine-enhanced beef

The objective of this study was to validate the use of lactic acid bacteria (LAB), acidified sodium chlorite (ASC), and lactic acid (LA) sprays as interventions to reduce transfer of *E. coli* O157:H7 and *Salmonella* Typhimurium from inoculated surfaces into the interior of the meat after processing in a simulated purveyor setting. Choice strip loins (longissimus lumborum muscles) intended for either mechanical blade tenderization (MT) or injection enhancement (EN) with brine after an aging period of 14 or 21 days at 4.4°C under vacuum were inoculated. For both processes, the samples treated only with water (control) consistently presented higher microbial counts in the internal surfaces than those samples treated with the interventions. Results from this study showed that the effectiveness of the interventions varied with different degrees of efficacy depending on the process (MT or EN),

the day in which the intervention is applied, or the sampling location within the piece. In general, *Salmonella*- and *E. coli* O157:H7-inoculated samples subjected to MT and treated with LAB and LA presented the lowest internal pathogen counts. Similarly, those pieces subjected to brine enhancement presented the lowest transfer when treated with ASC and LAB (in the case of *E. coli* O157:H7) and LA and ASC (in the case of *Salmonella*). The application of antimicrobials by purveyors prior to mechanical tenderization or enhancement of steaks could increase the safety of these types of products.
<http://www.ingentaconnect.com/content/iafp/jfp/2010/00000073/00000012/art00003>

Viability of *Bacillus licheniformis* and *Bacillus thuringiensis* spores as a model for predicting the fate of *Bacillus anthracis* spores during composting of livestock mortalities

Safe disposal of livestock mortalities and contaminated manure is essential for the effective control of infectious diseases outbreaks. Composting has been shown to be an effective method of disposal, but no information exists on its ability to contain diseases caused by spore-forming bacteria such as *Bacillus anthracis*. Hence, this Canadian study utilised duplicate composters, each containing 16 cattle mortalities (final capacity 85,000 kg). Spores of *B. licheniformis* and *B. thuringiensis* were mixed with autoclaved feedlot manure and either placed in sterile vials or into porous nylon bags. Temperatures in Composter 1 were slightly higher than in Composter 2 by up to 10°C. Composting reduced the number of viable spores isolated from *Bacillus* spp. as compared to the inoculated control that was held at room temperature. The number of viable spores of both *Bacillus* species declined in Composter 1 after 230 days, however, viable spores declined then increased again in Composter 2. This study indicates that spore viability was reduced in Composter 1 by exposure to elevated temperatures over time. Different temperature profiles may explain why spores remained viable in Composter 2, but were largely

rendered non-viable in the Composter 1 structure. Under practical conditions, variation in composting microclimates may preclude the complete inactivation of *Bacillus* spores, including those of *B. anthracis* during composting. However, composting may still have merit as a method of biocontainment, reducing and diluting the transfer of infectious spores into the environment.
<http://aem.asm.org/cqi/content/abstract/AEM.01889-10v1>

FOOD SAFETY MANAGEMENT STRATEGIES

A concurrent diagnosis of microbiological food safety output and food safety management system performance: Cases from EU meat processing industries

Food processing companies are required to review their food safety management system (FSMS) performance to improve food safety. Performance is rated by checking compliance against pre-set requirements via audits/inspections. Food safety (FS) output is analysed by microbiological testing. This paper discussed the usefulness of a concurrent analysis of European FSMS performance and FS output using three meat-processing companies. The first analysis (FS) used *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., and *E. coli* and total viable counts (TVC) at ten sampling locations, covering both product and environmental samples. The second analysis (FSMS) used the measurement of the risk of FSMS performance in core activities using 51 indicators and corresponding levels of compliance descriptions. For the large beef meat processor, the FS output diagnosis showed high TVC but the high activity scores of their FSMS indicated that this problem could be solved by supplier measures. Likewise, for the medium-sized poultry meat processor, the FSMS diagnosis showed a clear dependency on suppliers. However, the FS output diagnosis revealed a broader contamination problem, and

additional measures such as a sanitation program, compliance to procedures and personal hygiene requirements could improve the results. The FS output diagnosis of the small lamb meat processor showed contamination problems (but no pathogens) corresponding with FSMS low activity levels in combination with the high-risk context. The diagnosis of both FSMS and FS could provide directions for improvement to move towards a more targeted microbial food safety management system.

[http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6S-518TDNX-2&_user=10&_coverDate=04%2F30%2F2011&_rdoc=33&_fmt=high&_orig=browse&_origin=browse&_zone=rslt_list_item&_srch=doc-info\(%23toc%235038%232011%23999779996%232733749%23FLA%23display%23Volume\)&_cdi=5038&_sort=d&_docanchor=&_ct=45&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=de1fee8e709e5dc2da4e4b550194b8cb&searchtype=a](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6S-518TDNX-2&_user=10&_coverDate=04%2F30%2F2011&_rdoc=33&_fmt=high&_orig=browse&_origin=browse&_zone=rslt_list_item&_srch=doc-info(%23toc%235038%232011%23999779996%232733749%23FLA%23display%23Volume)&_cdi=5038&_sort=d&_docanchor=&_ct=45&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=de1fee8e709e5dc2da4e4b550194b8cb&searchtype=a)

CHARACTERISING MICROBIAL SPOILAGE

Characterisation of psychrotrophic bacterial communities in modified atmosphere-packaged meat with terminal restriction fragment length polymorphism (TRFLP)

To overcome the limitations of bacterial culture methods for the characterisation of psychrotrophic bacterial communities in meat, this Finnish research project used a culture-independent, DNA-based 16S rRNA gene-targeted terminal restriction fragment length polymorphism (TRFLP) method. Characterization of the psychrotrophic bacteria communities was used to understand the microbial ecology of spoilage in modified atmosphere-packed (MAP) meats. Meat samples (80% of pork and 20% beef) packed under modified atmosphere containing 65% O₂, 25% CO₂, and 10% residual air were obtained from a commercial processing plant. A bacterial isolate library consisting of 100 Gram-positive

and 30 Gram-negative meat-associated bacterial strains was set up to identify bacterial species determined by TRFLP analysis in MAP minced meat at the end of the shelf life. Bacteria identified by the DNA-based method (TRFLP) were compared to bacteria identified using typical culture-based method for lactic acid bacteria (LAB). Both methods agreed that *Leuconostoc* spp. and *Carnobacterium* spp. prevailed in the LAB community in minced meat. Hence, the TRFLP results were shown to correlate with viable counts. The TRFLP method was found to be a relatively rapid and inexpensive method to characterize a large number of communities of psychrotrophic bacteria prevailing in MAP meat.

[http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T7K-51962NY-5&_user=10&_coverDate=01%2F05%2F2011&_rdoc=6&_fmt=high&_orig=browse&_origin=browse&_zone=rslt_list_item&_srch=doc-info\(%23toc%235061%232011%23998559996%232781734%23FLA%23display%23Volume\)&_cdi=5061&_sort=d&_docanchor=&_ct=37&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=7d3182fec723f17ebb43d3f9a3b584fd&searchtype=a](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T7K-51962NY-5&_user=10&_coverDate=01%2F05%2F2011&_rdoc=6&_fmt=high&_orig=browse&_origin=browse&_zone=rslt_list_item&_srch=doc-info(%23toc%235061%232011%23998559996%232781734%23FLA%23display%23Volume)&_cdi=5061&_sort=d&_docanchor=&_ct=37&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=7d3182fec723f17ebb43d3f9a3b584fd&searchtype=a)

DETECTION METHODS

Detection of *E. coli* in meat with an electrochemical biochip

The detection of pathogenic and hygienically relevant microorganisms is important for health and food safety. Rapid and sensitive bacterial detection methods used in medicine are of interest to the food industry. Detection of *E. coli* in meat is an important indicator for the hygienic status of food-producing companies. In this German study, sample pre-treatment and electrochemical detection of RNA-DNA were combined for fast and sensitive detection of food-spoiling bacteria. Pork and beef samples were either naturally or artificially contaminated by *E. coli* and all samples were enriched by incubation in medium. A lysis treatment of samples resulted in efficient cell disruption and high total RNA yields. Together with optimization of enrichment

time, this ensures high sensitivity of electrochemical measurements on the biochips. A short enrichment period and the triple-cell lysis regimen in combination with electrochemical biochip measurement were tested with 25 meat samples. The analysis (5 hours of enrichment, triple lysis, and biochip detection) has a lower limit of detection of 1 CFU of *E. coli* per ml within a total time for analysis of 7 hours. Hence, with an improved sample treatment and higher sensitivity an efficient method for detection of pathogenic bacteria was developed.

<http://www.ingentaconnect.com/content/iafp/ifp/2010/00000073/00000011/art00010>

Factors that interfere with the detection of low numbers of *E. coli* O157:H7 by culture isolation and rapid methods

Some industry testing programs require the ability to detect *E. coli* O157:H7 in samples of beef trim or ground beef at levels as low as 1 cell/375 gram. A protocol was studied for generating a control inoculum for verification testing at this low concentration and evaluated its use. During the initial phase of the studies, it was noted that two factors may contribute to inconsistent *E. coli* O157:H7 detection results. One was the concentration of background organisms present (APC) and the other was the lean-to-fat ratio of the trim or ground beef being tested. When 5 cells of *E. coli* O157:H7 were inoculated into 375 gram of ground beef, no detection method was 100% effective. Detection by culture isolation and two commercial rapid assays detected *E. coli* in 94%, 92%, and 92% of samples inoculated (range 1 to 9 cells), respectively. At APC concentrations below 6 log CFU/g, the rapid methods detected all beef trim samples inoculated with *E. coli* O157:H7. However, at higher background bacteria levels, the commercial assay efficacy decreased. Increased fat content was observed not to interfere with detection of *E. coli* O157:H7. The results provide some guidelines for lower limits of detection of microbial contamination or when the samples being tested are of varying fat percentages.

<http://www.ingentaconnect.com/content/iafp/ifp/2010/00000073/00000012/art00004>

MICROBIAL PREVALENCE

Prevalence of *Clostridium difficile* in retailed meat in The Netherlands

A large proportion of community-acquired human *C. difficile* infections (CA-CDI) may not be linked to antibiotic therapy, older age, multiple infections or previous hospitalization. Other possible community sources for CA-CDI include animals and food, and therefore a study on the prevalence of *C. difficile* in meat was performed. Samples of different meat species, including raw beef, pork, calf, lamb and chicken meat were collected from the retail trade and analyzed for the presence of *C. difficile* using methods that included selective enrichment in broth, subsequent alcohol shock-treatment and plating onto selective medium. Isolates were tested for the presence of toxin genes and were typed using ribotyping (a method of genetic fingerprinting). Of the 500 samples tested, eight (1.6%) were positive for the presence of *C. difficile*: one from lamb (6.3%) and seven from chicken meat (2.7%). The isolated strains from meat belonged to ribotypes different from those that are frequently found in patients with CDI in the Netherlands, except for one ribotype which was found in one chicken meat sample. This study shows that there is a small potential for transmission of *C. difficile* to humans via the food chain. Rates of contaminated food product varied strongly and different *C. difficile* types were found in different countries. However, until now there are no documented cases of infection that resulted from eating food that contained this bacterium.

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T7K-51FXR2H-4&_user=10&_coverDate=01%2F05%2F2011&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&_view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=7bbd819564358e169c5e77653f6a6bce&searchtype=a

NANOTECHNOLOGY

Reduction of the spoilage-related microflora in absorbent pads by silver nanotechnology during modified atmosphere packaging of beef meat

This Spanish research project developed silver-based antibacterial hybrid materials by *in situ* reduction of silver nitrate (1%) adsorbed on cellulose fibres by thermal and UV treatments. Microscopy revealed that the silver nanoparticles were dispersed and regular in shape. Migrated silver ion concentrations reached 60 ppm in beef meat exudates. The ability of the silver-loaded absorbent pads to lower microbial contamination of exuded fluids was studied during storage of beef meat in modified atmosphere packaging. Cellulose-silver hybrid materials reduced the levels of the major microbial groups (APC, LAB, *Pseudomonas* spp., and Enterobacteriaceae) present in the absorbent pads by an average of 1 log CFU/g during the entire storage period. The levels of total aerobic bacteria and *Pseudomonas* spp. were significantly reduced in the presence of silver ions, whereas LAB were less sensitive and not significantly affected. Enterobacteriaceae levels remained under the detection limit when silver was present. Neither the colour of the meat nor the microbial loads were markedly affected by the presence of the silver-based antimicrobial hybrid materials.

<http://www.ingentaconnect.com/content/iafp/ifp/2010/00000073/00000012/art00016>

Carbohydrate nanoparticles for prolonged efficacy of antimicrobial peptides against *Listeria monocytogenes*

Carbohydrate nanoparticles were created to prolong the efficacy of an antimicrobial peptide, nisin, against a pathogen, *Listeria monocytogenes*. Phytoglycogen (PG)-based nanoparticles (developed from corn starch) were developed as the carriers of nisin. The goal of this USA research was to minimise the loss of the peptide during storage as well as

develop an effective release in the presence of bacteria. To evaluate the prolonged nisin efficacy, preparations containing nisin and PG derivatives were loaded into an agar model (mimicking nisin depletion at the nutrient surface). The residual inhibitory activities of preparations against *L. monocytogenes* were monitored during 21 days of storage at 4°C. The results showed that all PG derivatives led to the prolonged retention of nisin activity and *L. monocytogenes* growth inhibition. Results indicate both electrostatic and hydrophobic interactions are the driving forces of nisin adsorption, and the surface structure of the nanoparticle also affects nisin loading and retention during storage.

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T3D-51JXFF1-2&_user=10&_coverDate=11%2F27%2F2010&_alid=1631551764&_rdoc=1&_fmt=high&_orig=search&_origin=search&_zone=rslt_list_item&_cdi=4944&_sort=r&_st=13&_docanchor=&_view=c&_ct=13&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=e828ca7573bd5abcdeefcf9c5e2cd975&searchtype=a

INDUSTRY VACCINE TRIAL

Cargill encouraged by preliminary results from beef cattle vaccine trial

Initial results from Cargill's 2010 *E. coli* O157:H7 vaccine trial involving 85,000 head of beef cattle in the USA, based upon sampling both cattle at feedlots and meat, have been encouraging. Of the 85,000, nearly 60,000 head of cattle received two doses of the vaccine produced, one dose upon arrival at the feedlot and one dose approximately 90 days prior to harvesting. The remaining cattle received a single dose and served as buffers prior to and following those cycling through the feedlots that received two doses. By providing buffers, Cargill established the scientific controls required to test the effect of whole feedlot vaccination under commercial conditions. Because of the favourable immune system response to the vaccine, and the cattle had no adverse reaction. There will be a

second vaccine trial, possibly in the summer of 2011. There were numerous factors that were found to influence the potential effectiveness of vaccine for reducing naturally, randomly-occurring *E. coli* O157:H7 in beef cattle, including weather, geography, seasonality, animal and herd care and management, vaccine dosage and others, which creates a challenge in replicating the prior trial. Furthermore, it was noted that there was a low level of *E. coli* O157:H7 in the beef being produced from the non-vaccinated (control group) cattle during the time the vaccinated cattle were being harvested, potentially influencing the significance of the data currently being analysed by independent researchers. These researchers are trying to better understand the long-term efficacy of the vaccine in reducing in *E. coli* O157:H7 in beef. Cargill stated that they hope this research will lead to validating the potential value of vaccine as another food safety tool for beef production. <http://www.cargill.com/news-center/news-releases/2010/NA3036619.jsp>

PROCESSING PLANT HYGIENE

European study indicates recycled hot water for carcass treatment is as effective as hot potable water

Using hot recycled water as a decontamination technique for meat carcasses is as effective as hot potable water, according to the European

Food Safety Authority (EFSA). For carcass decontamination purposes, only potable water is currently allowed in the EU. However, recycling (reusing after reheating) of the water used for carcass decontamination has been practiced in some countries (e.g. Canada, Denmark) for environmental and energy-preserving reasons. In this research, potential microbiological and abiotic risks for carcasses associated with recycled hot water decontamination, and related control options, were considered. It was concluded that the decontamination efficacy of recycled hot water does not differ significantly from that of hot potable water. With recycled hot water, only microbiological risks associated with heat-resistant bacterial spores (*Clostridium botulinum*, *C. perfringens*, *C. difficile* and *Bacillus cereus*) are relevant. These risks can be controlled through ensuring that recycled hot water is verifiably subjected to such reheating and frequency of renewal regimes which ensure that the microbiological risk in recycled water meets the same HACCP criteria as hot potable water. It was recommended that further research should occur on the presence and potential accumulation of bacterial spores in the water for decontamination of carcasses of all animal species, and on the potential presence and accumulation of residues of veterinary drugs and other chemical contaminants in the hot recycled water for decontamination of poultry carcasses.

<http://www.efsa.europa.eu/en/efsajournal/pub/1827.htm>

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